Chmielarz et al. Annals of Forest Science (2022) 79:3
https://doi.org/10.1186/s13595-022-01121-3

INRAE
Annals of Forest Science

RESEARCH PAPER Open Access

Desiccation does not increase frost resistance of pedunculate oak (*Quercus robur* L.) seeds

Paweł Chmielarz, Jan Suszka and Mikołaj Krzysztof Wawrzyniak*

Abstract

Key message: Decreasing acorns moisture content does not significantly increase the frost resistance of pedunculate oak seeds. Slight reduction in acorn moisture content below the relatively high, optimal level decreased seed survival at temperatures below − 5 °C. The limiting temperature for pedunculate oak's acorns below which they lose their ability to germinate is about − 10 °C.

Context: Seed moisture content plays an important role in successful seed storage of many species, as desiccation increases frost resistance; however, oak seeds tolerate desiccation only to a very small extent.

Aims: In our study, we examined the impact of decreasing moisture content in acorns of pedunculate oak (*Quercus robur* L.) on their frost resistance (below − 3 °C) and the growth of seedling derived from frozen seeds.

Methods: Germination and seedling emergence of individual seeds, as well as the dry mass of their 3-month-old seedlings, were measured after acorn desiccation (24–40%, fresh weight basis) and desiccation followed by freezing at temperatures from − 3 °C to − 18 °C for 2 weeks.

Results: Decreasing acorns moisture content did not significantly increase the frost resistance of pedunculate oak seeds. The lowest temperature at which at least half seeds remain viable was − 10 °C. Slight acorns desiccation had only a small positive effect on seeds frozen below − 11 °C (down to − 13 °C), but in this case (acorn moisture content of 33%), low germinability after freezing made storage uneconomic because of the high mortality of seeds. Germinated seeds after desiccation and freezing showed no significant difference in later growth.

Conclusion: Fresh pedunculate oak seed can survive freezing temperature down to − 10 °C and produce good quality seedlings. Temperatures around − 11° to − 13 °C are near lethal to acorns and significantly reduce their viability. Overall, desiccation does not increase their frost resistance; therefore, in practice, it is important to keep acorns during a cold storage in the highly hydrated state.

Keywords: Seeds storage, Oak, Desiccation, Frost resistance, Germination

* Correspondence: mikwaw@man.poznan.pl
Handling editor: Erwin Dreyer.
Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland

1 Introduction

Based on the response to desiccation, seeds can be divided into three categories: orthodox, recalcitrant (Roberts 1973) and intermediate (Ellis et al. 1991). Orthodox seeds can tolerate desiccation to low moisture content < 5% on a fresh weight basis (water potential between − 35 and − 40 MPa) (Chmielarz 2010a; Roberts 1973), with no decrease in their germinability. Recalcitrant (desiccation–sensitive) seeds are killed by drying below a relatively high level of moisture content ≤ 20–50% (Pritchard 2004; Suszka and Tylkowski 1980). The range of tolerance in recalcitrant seeds is narrow and usually limited to approximately 20% moisture content (− 12 MPa); however, the threshold is species-specific. Intermediate seeds tolerate desiccation to moisture contents of ca. 7–12% but lose viability rapidly during storage at colder temperatures (Ellis et al. 1991; Chmielarz 2007; Hong et al. 1998).

Mature recalcitrant seeds have a relatively high water content and do not exhibit a long quiescent period (Berjak et al. 1999). These seeds remain metabolically active throughout the post-maturation period and up to the beginning of the germination process. Therefore, recalcitrant seeds are usually short-lived and impossible to store using conventional storage methods (Subbiah et al. 2019). The direct cause of recalcitrant seed-tissue death at temperatures below 0 °C is the formation of ice crystals (Chmielarz 1997).

Oak seeds usually do not tolerate a desiccation below 30–40% acorn moisture content (Gordon 1992; Chmielarz and Walters 2007). However, desiccation sensitivity depends on species-related physiology. Seeds of the white oaks—section *Quercus* (synonyms *Lepidobalanus* and *Leucobalanus*)—mature in 6 months and are very sensitive to desiccation and difficult to store successfully for more than 1–2 years. Seeds of the black oak group—section *Lobatae* (synonym *Erythrobalanus*)—mature in 18 months and are more desiccation tolerant than those of the white oak group (Schroeder and Walker 1987; Gosling 1989; Connor and Sowa, 2002, 2003; Chmielarz and Walters 2007). Earlier studies showed that the critical moisture content for pedunculate oak (*Quercus robur,* section *Quercus*) seeds was approximately 40%, below which viability declined rapidly (Suszka and Tylkowski 1980; Gosling 1989; Poulsen and Eriksen, 1992).

Snow cover is an important protection against abiotic stresses such as frost desiccation and cold air. Winter conditions are a major factor influencing seedling establishment (Renard et al. 2016). Effective protection of the ground vegetation from low temperatures (− 30 °C or lower) during winter depends on the duration and thickness of the insulating snow cover (Domisch et al. 2018). However, due to climate changes snowdays and snow cover duration decrease in temperate zones, which is especially apparent in mountain ecosystems (Kreyling and Henry 2011; Yeo et al. 2017). For example, under the RCP 4.5 and 8.5 emissions scenarios, a 49–95% reduction in snow cover in the northeastern USA is projected for 2100 (Reinmann et al. 2019). Without insulation, many seeds and seedlings can be affected by low winter temperatures and desiccation, which in result can limit their growth or in more severe cases lead to their death.

Recalcitrant seeds can be stored only under non-freezing temperatures and appropriate relative humidity levels for a short time, from several months to 1–2 years (Tompsett 1998; Suszka et al. 1996). During such storage, the seeds may either germinate or be affected by pathogens (in acorns, mainly by the fungus *Ciboria batschiana* (Zopf) N.F. Buchw.) Schröder 2002; Suszka 2002; Berjak and Pammenter, 2000). To date, the optimal storage conditions identified for pedunculate oak acorns are a moisture content of 40% and a temperature of − 3 °C in containers that are not tightly closed. However, over 2 years, pedunculate oak seeds rapidly lose their ability to germinate (Suszka et al. 1996). Because of the infrequent occurrence of mast years in Europe (usually 3–8 years), a longer period of safe acorn storage in controlled conditions is needed (Suszka et al. 1996). Suszka et al. (1996) reported that pedunculate oak acorns (at moisture content 42–45%) can be stored for 1.5–2 years without loss of seeds germinability and seedling emergence at temperature − 1° or − 3 °C.

Data available in the literature regarding seed frost resistance are usually related to several months of acorn storage. In our research, we attempted to answer the question of how acorn moisture content influences seed freezing sensitivity, for better understanding the survival of pedunculate oak seeds during cold, snowless winters and possibly increasing the safe seeds storage in controlled conditions. We hypothesized that a reduction in acorn moisture content can increase frost resistance of pedunculate oak seeds. We examined, for the first time, the sensitivity of seeds to freezing temperature on the basis of individual acorn germination, seedling emergence and their growth in correlation with their individual moisture content.

2 Materials and methods

2.1 Seed material

Acorns from two Polish provenances of *Quercus robur* L., Czmoń (later in the study referred to as seed lot A)—forest subdistrict (District Babki: 52°18′41.8″N 16°56′33.1″E)—and Siedlisko (later in the study referred to as seed lot B)—forest subdistrict (District Nowa Sól: 51°46′48.2″N 15°43′18.5″E), were collected in the middle of October in the western part of Poland, 100 km apart, in 2018 for seed lot A and in 2017 for seed lot B. After collection from the ground, acorns were transported to the Institute of Dendrology PAS in Kórnik, where they were preliminarily stored at 3 °C in containers that were not tightly closed. Acorns of seed lot A were stored in these conditions for 8 months until the experiment started. Acorns of seed lot B were preliminarily stored in similar conditions for 1 month. The mean moisture content of the acorns upon delivery was 37.7% of fresh weight for seed lot A and 40.8% for seed lot B. During preliminary storage, the acorns were not affected by *Ciboria batschiana*; thus, no thermotherapy was required before the experiment.

2.2 Acorn drying

Acorns of both seed lots were partially dried to obtain different moisture content levels at room temperature (approximately 20 °C) for up to 29 days. During drying, acorns were mixed regularly to reach uniform drying. Acorns from seed lot B collected in 2017 were dried in an open space in an air stream forced by a fan. This caused fast acorn desiccation, and consequently, some of the acorns showed a cracked pericarp. The final mean moisture content of these dried acorns after 29 days remained at 24.3%. Acorns collected in 2018 (seed lot A) were dried in a closed room with a higher air humidity reaching, after 29 days, a mean moisture content of 29.8%. The conditions used in the last method of acorn drying prevented the acorns from cracking (smaller spread in acorn moisture content at the final stage of drying of seed lot A, Fig. 1). Overall, – 3278 acorns were used for seed lot A, and 4400 acorns were used for seed lot B.

2.3 Determination of the moisture content of individual acorns

After each drying period, 30–90 individual acorns (seeds and pericarp) from each seed lot were cut into apex and distal parts. The corresponding distal parts of the acorns were used to determine the moisture content after oven drying at 103±2 °C for 17 h. The results were used to create a standard curve, which allowed us to determine a total acorn moisture content (Appendix Fig. 4). Moisture content was calculated on a fresh weight (FW) basis and expressed in percentage.

The average moisture content of drying samples of acorns was determined for three replicates of 10 halves of acorns using a Sartorius MA37 weight dryer (120 °C for 20 min) calibrated according to a classical oven-drying method (103±2 °C for 17 h).

2.4 Acorn freezing

After desiccation, acorns were packed in polyethylene bags (300 acorns/bag) and frozen directly down to temperatures of – 3°, – 5°, – 7°, – 9°, – 11° or – 13 °C for seed lot A and down to – 3°, – 10° or – 18 °C for seed lot B. Frozen samples were kept at each temperature for 2 weeks. The control treatments for the two seed lots were stored at a temperature of 3 °C for two weeks.

2.5 Germination/seedling emergence test

An earlier described test of pedunculate oak was applied (Giertych and Suszka 2010; Giertych and Suszka, 2011). Before sowing acorns into the medium, the distal end (hilum end) of each acorn was cut off, and the remaining part (apex part) was planted with the radicle end

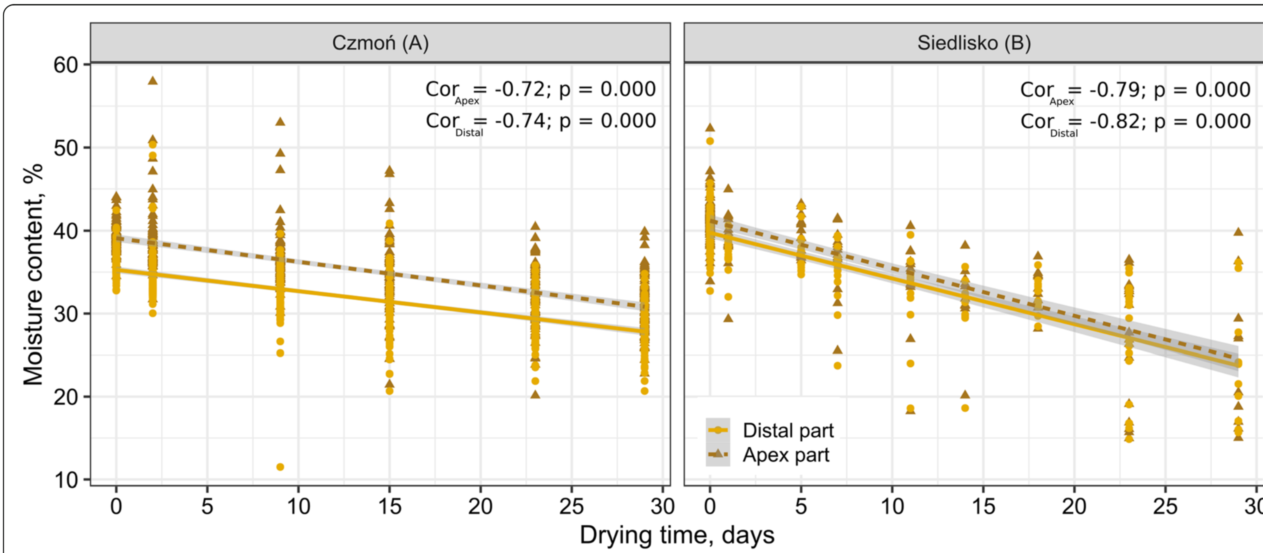

Fig. 1 Effect of *Quercus robur* acorn drying time, up to 29 days, on the moisture content of the apex and distal parts. Czmoń (**A**) seed lot—acorns collected in 2018—and Siedlisko (**B**) seed lot—acorns collected in 2017. Cor—Spearman's correlation coefficient

upwards. Seeds with a radicle at least 5 mm long were considered germinated.

Individual acorns (their apex part) were sown vertically in a one 1-l container filled with a moist mixture (1:1, v/v) of quartz sand (< 1 mm fraction) and sieved peat (pH 5.5–6.5) and covered with a lid. In experiments with seed lot A, 90 acorns, and for seed lot B, 100 acorns, were sown in 3 containers for each treatment of the experiment. The thermal conditions for germination and seedling emergence within an environmental chamber were optimized at 20 °C and a 16/8 h photoperiod under a light intensity of 60 μmol m^{-2} s^{-1} provided by a fluorescent lamp (Fluora, Osram™) (Suszka and Tylkowski 1980). Germinating seeds were left in the same containers for 4– weeks to monitor seedling emergence (Suszka and Tylkowski, 1980). When the shoot was at least 5 cm long, the seedling was classified as emerged. After 2–3 weeks of seedling growth the lids were removed to decrease the air humidity for growing shoots and to prevent fungal development on the small leaves.

2.6 Seedling biomass

After 3 months of further cultivation of seedlings under the same light and thermal conditions, the seedlings finished their growth, having 4–6 leaves and a terminal bud on the shoot top. Height, root collar diameter and dry mass (above- and belowground) were recorded on these three months old seedlings (Suszka et al. 1996). The dry mass was determined after drying at 105 °C for 17 h.

2.7 Statistical analysis

All statistical analyses were conducted in R software (R Core Team 2021), and because of differences in experimental design, seed lots were analysed separately. Dataset used for analysis can be found in Figshare repository (Wawrzyniak et al. 2021). The correlation between the moisture contents of the apex part and distal end of acorns was derived from linear regression analysis. Prior to analysis, databases were cleaned using the *tidyverse* package (Wickham et al. 2019). Outliers were identified using the interquartile range criterion and removed from the database to obtain a better model fit, expressed by the Akaike information criterion (AIC) value of the model. The effect of acorn drying time on germination and seedling emergence was evaluated using a generalized linear model (GZLM) with a binomial distribution (Ranal and de Santana 2006). In the case of overdispersion, the models were fit using a quasibinomial distribution (Crawley 2013). For the post hoc test, Tukey's method was used with adjusted p values. Post hoc tests were performed using the *emmeans* package (Lenth 2022). A GZLM was also used to calculate the seedling emergence probability at a given moisture level under different temperatures. The GZLM was fit using a binomial distribution with a logit-link function. The model was chosen depending on the AIC. To examine the goodness of fit of the model, Mc Fadden's R squared and the area under the curve (AUC) were analysed (Crawley 2013). For the linear regression models used in growth analysis, outliers were detected and removed using Cook's distance estimates (Field et al. 2012). All data were log transformed to reach the normality of the distribution and stabilize variance homogeneity. Model assumptions were tested using Shapiro-Wilk's test and Levene's test. Additionally, adherence to the assumptions was checked using plot visualization. For all data visualization, the *ggplot2* package was used (Wickham et al. 2016)

3 Results

3.1 Effect of acorn drying on the moisture content of the apex and distal parts

The average moisture content of the distal parts was lower (non-desiccated: mean moisture content 36%, dried 29 days: mean moisture content 28%) than that of the apex part (non-desiccated: mean moisture content 39%, dried 29 days: mean moisture content 32%) in both examined seed lots (Fig. 1). After a longer period of drying of acorn samples, divergence of extreme values of the individual moisture content of acorns was observed for both the distal and apex parts within the samples. This was particularly evident from day 15 for seed lot A and from day 23 for seed lot B. High variation of individual acorn moisture content shows how different can be mean moisture content after the drying period. Even after a month of desiccation, some acorns retained high moisture content (ca. 40%). Acorns drying was very uneven and restrained by pericarp.

3.2 Effect of acorn moisture content on freezing sensitivity

Generally, seeds desiccation resulted in decreased germination and emergence at almost all tested freezing temperatures in both seed lots (Table 1 and Appendix Table 4). However, an exception was observed for a slight drying of acorns. For example, undried seeds from seed lot A stored at − 7 °C germinated at a significantly lower frequency (40%) than seeds dried for 2 days (90%), with seedling emergence frequencies of 36% and 86%, respectively. Seeds frozen in acorns at − 9 °C that were not desiccated germinated at a frequency of 19% (emergence of 17%). Acorns desiccated for 2 days frozen at − 9 °C exhibited an increased germination frequency of 70% (emergence of 67%). Prolonged desiccation (more than 18 days) decreased germination significantly in all tested temperatures (Table 1). Protective effect of drying from subzero temperature was observed in − 11° and − 13 °C. Seeds of non-desiccated acorns frozen at − 13 °C

Table 1 Germination and seedling emergence of *Quercus robur* seeds desiccated in acorns for up to 29 days (non-frozen)—control and seeds desiccated and then frozen for 2 weeks at − 3°, − 5°, − 7°, − 9°, − 11° or − 13 °C (seed lot A) and at − 3°, − 10° or − 18 °C (seed lot B). One-way ANOVA; means marked with the same letter are not significantly different at $p < 0.05$ according to Tukey's test. Means±SEs.Acorns moisture content in % of fresh weight. Full model results can be found in Appendix Table 4

	Drying time, days (av. moisture content, %)	Non-frozen	− 3 °C	− 5 °C	− 7 °C	− 9 °C	− 10 °C	− 11 °C	− 13 °C	− 18 °C
	Germination, %									
Seed lot A	0 (37.7)	98±1.11 a	93±3.85 a	92±2.22 a	40±5.77 b	19±4.01 c	-	2±1.11 c	2±1.11 b	-
	2 (36.5)	96±2.22 a	98±1.11 a	100±0 a	90±1.92 a	70±5.09 a	-	19±4.44 b	4±2.94 b	-
	9 (34.1)	91±2.94 a	97±1.92 a	94±2.94 a	91±2.94 a	80±10.72 a	-	28±2.94 ab	13±5.09 ab	-
	15 (32.9)	93±3.33 a	86±2.22 ab	89±4.01 a	82±4.01 a	74±4.01a	-	41±4.84 a	21±2.22 ab	-
	23 (31.0)	76±8.01 ab	76±2.94 b	63±5.09 b	57±1.92 b	53±3.33 ab	-	34±1.11 ab	27±6.67 a	-
	29 (29.8)	56±7.78 b	41±1.11 c	51±6.76 b	50±1.92 b	28±4.01 bc	-	23±5.77 ab	16±4.01 ab	-
	Seedling emergence, %									
	0 (37.7)	93±1.92 a	91±2.94 a	89±2.22 a	36±4.01 c	17±1.92 b	-	2±1.11 c	1±1.11 a	-
	2 (36.5)	87±3.85 ab	96±2.94 a	100±0 a	86±2.22 a	67±5.09 a	-	16±4.44 bc	4±2.94 a	-
	9 (34.1)	867±3.85 ab	91±4.01 a	83±3.85 a	79±5.88 ab	74±10.94 a	-	23±5.09 ab	11±4.84 a	-
	15 (32.9)	89±1.11 a	63±3.33 b	79±4.01 a	64±7.29 b	58±2.22 a	-	34±4.01 a	12±2.22 a	-
	23 (31.0)	64±8.89 b	54±1.11 b	44±4.01 b	34±4.01 c	29±4.84 b	-	17±1.92 abc	14±4.84 a	-
	29 (29.8)	31±7.78 c	22±4.84 c	28±2.94 b	29±4.01 c	13±3.33 b	-	7±1.92 c	2±1.11 a	-
Seed lot B	Germination, %									
	0 (40.8)	96±2.31 a	88±2.83 ab	-	-	-	6±3.46 b	-	-	0
	1(38.2)	92±4.90 ab	91±1.00 a	-	-	-	48±2.83 a	-	-	0
	5(38.8)	89±3.00 abc	84±4.00 abc	-	-	-	65±3.42 a	-	-	0
	7(35.7)	93±1.91 ab	78±4.2 abcd	-	-	-	62±6.00 a	-	-	0
	11(32.7)	76±1.63 bcd	73±7.2 bcde	-	-	-	65±3.42 a	-	-	0
	14(31.1)	75±4.43 bcd	67±3.42 cde	-	-	-	52±5.42 a	-	-	0
	18(32.6)	73±3.42 bcd	57±6.61 def	-	-	-	50±7.39 a	-	-	0
	23(28.7)	68±9.09 cd	54±5.29 ef	-	-	-	47±5.97 a	-	-	0
	29(24.3)	53±3.00 d	41±1.91 f	-	-	-	13±7.55 b	-	-	0
	Seedling emergence, %									
	0 (40.8)	90±2.00 a	85±1.91 a	-	-	-	6±3.46 b	-	-	0
	1(38.2)	87±5.51 ab	88±2.83 a	-	-	-	46±3.46 a	-	-	0
	5(38.8)	86±4.16 ab	81±3.79 ab	-	-	-	61±2.52 a	-	-	0
	7(35.7)	85±5.74 ab	74±5.77 ab	-	-	-	56±5.16 a	-	-	0
	11(32.7)	72±1.63 abc	65±5.26 bc	-	-	-	62±2.58 a	-	-	0
	14(31.1)	70±2.00 abc	62±3.46 bc	-	-	-	47±5.26 a	-	-	0
	18(32.6)	66±4.16 bc	50±3.46 cd	-	-	-	45±7.90 a	-	-	0
	23(28.7)	61±8.06 c	52±5.66 cd	-	-	-	40±4.32 a	-	-	0
	29(24.3)	49±4.12 c	38±2.58 d	-	-	-	8±4.90 b	-	-	0

showed a germination frequency of 2%. Temperature of − 18 °C has been proven to be lethal; frozen seeds did not germinate or emerge, regardless of whether the acorns were undried or desiccated.

The probability of seedling emergence of non-frozen seeds above 38% of moisture content was 100% (Fig. 2 and Table 2). Acorns with a moisture content of 30.7–30.9% cooled to − 3 or − 5 °C had a seedling emergence probability of 75% (in the 3rd quartile) (Table 3). The probability of seedling emergence from acorns desiccated before freezing at − 3°, − 5°, − 7°, or − 9 °C to a moisture content range of 25.4-26.4 was only 25% (1st quartile) (Table 3). The probability of obtaining seedlings from acorns frozen for two weeks at − 18 °C was 0% (seed lot B) (Fig. 2B).

3.3 Effect of acorns freezing on the growth of the 3-month-old seedling

A significant effect of acorn drying on the dry mass of the 3-month-old seedlings was observed regardless of

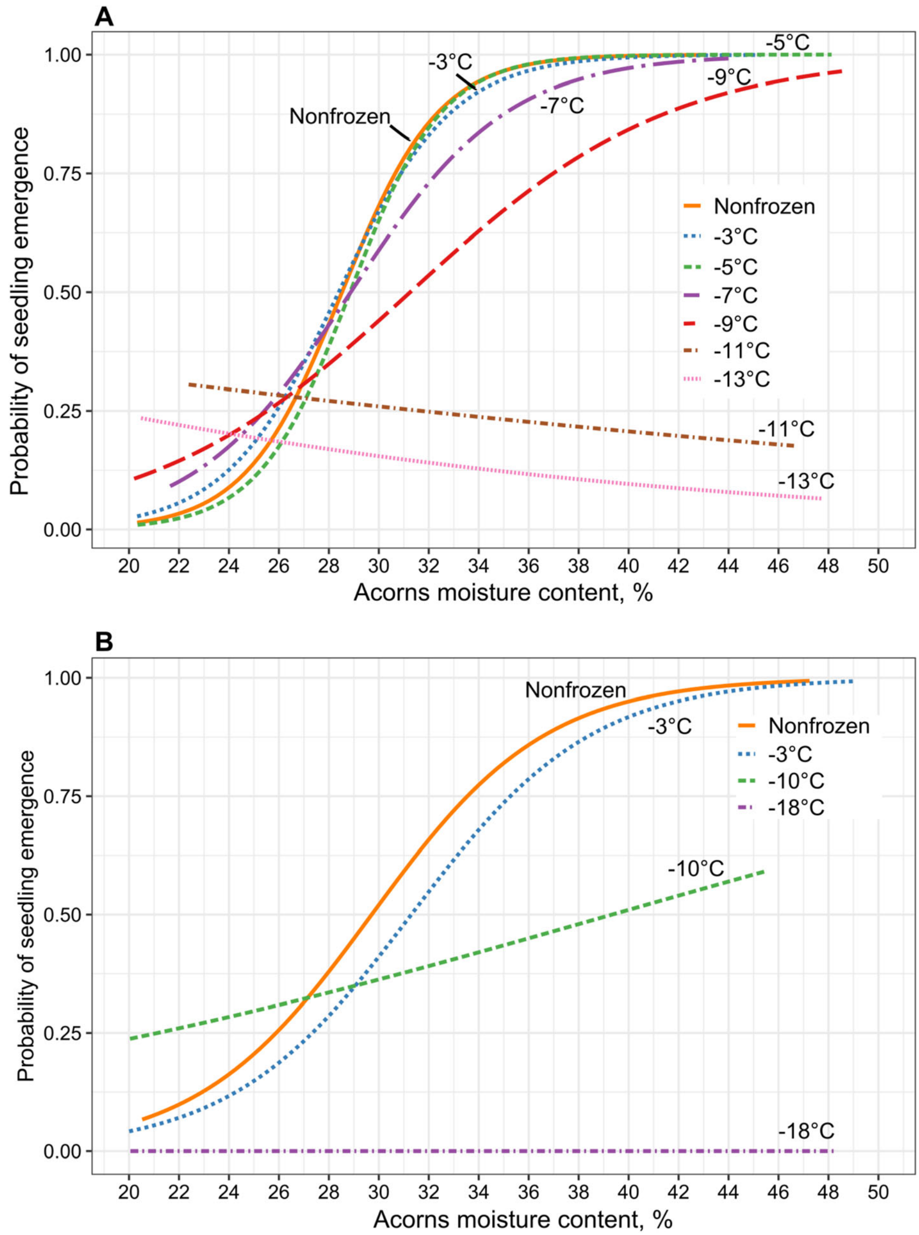

Fig. 2 Probability of *Quercus robur* seedling emergence depending on acorn moisture content and freezing temperature, i.e., − 3°, − 5°, − 7°, − 9°, − 11° or − 13 °C (seed lot **A**) and − 3°, − 10°, or − 18 °C (seed lot **B**), for 2 weeks. Non-frozen seeds—stored at 3 °C—were used as the control. Logistic regression analysis, model parameters can be found in Table 2

the freezing temperature used ($p < 0.05$, Fig. 3). The dry mass of seedlings decreased as acorns reduce their moisture content. There was no significant effect of temperature on plant dry mass, which in general was characterized by high variability (Fig. 3). However, the smallest seedlings with the lowest dry mass were obtained from seeds desiccated and frozen at the lowest temperature, − 11 °C or − 13 °C.

4 Discussion

4.1 Freezing sensitivity

Our results clearly demonstrated that relatively high levels of acorn moisture content (40%) previously described for stored seeds of pedunculate oak (Suszka et al. 1996), was the best moisture content for frozen acorns in our experiments. The safe cooling of seeds, down to − 7 °C, required a reduction in the initial acorn

Table 2 Model parameters for logistic regression. Seedling emergence of *Quercus robur* of different moisture content (MC) and interaction with tested temperature – 3°, – 5°, – 7°, – 9°, – 10°, – 11° – 13° or – 18 °C. (A) seed lot A; (B) seed lot B

	Variable	df	X^2	*p* value
Czmoń	Acorn moisture content	1	139.95	0.000
	Freezing Temperature	6	454.76	0.000
	AMC x FT	6	249.08	0.000
	Model specifics			
	Model X^2	1481.79		
	R^2 *Mc Fadden*	0.36		
	AUC	0.878		
	N	3001		
Siedlisko	Acorn moisture content	1	37.99	0.000
	Freezing Temperature	4	429.00	0.000
	AMC x FT	3	150.09	0.000
	Model specifics			
	Model X^2	2541.85		
	R^2 *McFadden*	0.44		
	AUC	0.896		
	N	4171		

moisture content by drying between 2 and 14 days. However, Chmielarz (1997) did not find – 7 °C to be a destructive temperature during cooling of pedunculate oak acorns with higher moisture content levels of 42–43%, cooling of 1 °C/min down to – 15 °C and maintenance at this temperature for 15 min. Seeds cooled further to – 9 °C, – 11 °C and – 13 °C remained viable (seedling emergence of 42–72%) (Chmielarz 1997). The examined exotherms (ice crystallization and tissue death) in this earlier experiment appeared in different acorns at temperatures of – 8° and – 10 °C (a thermocouple was placed between cotyledons). In our experiment, due to summer drought, the collected acorns were relatively dry (initial moisture content of 37.7%). After being frozen for 2 weeks at – 11 °C or – 13 °C, they germinated and a gave rise to seedlings at a lower level at these temperatures in comparison with those of acorns in our previous experiment with a higher moisture content of 42–43% (Chmielarz 1997). This freezing sensitivity will probably change for seeds stored for longer periods. It was also noticed that unfavourable weather conditions during seed maturation limit their storability (Guerrant et al. 2004). Acorns with a lower moisture content during collection are more sensitive, and their germination decreases more rapidly after longer storage under classic conditions (– 3 °C) (personal communication).

This shows that the frost resistance of pedunculate oak seeds depends on different factors that affect seeds before storage. Comparing the results for freezing temperatures of – 9 °C, – 10 °C and – 11 °C in the present

Table 3 Modelled quartiles of acorn seedling emergence probabilities as a function of acorn moisture content and storage temperature. See Table 2 for model parameters

Seed lot	Freezing temperature	Acorns moisture content, %			
		P25	P50	P75	P95
Czmoń (A)	Nonfrozen	26.4	28.5	30.7	34.2
	– 3 °C	25.9	28.4	30.9	35.1
	– 5 °C	26.8	28.9	30.9	34.3
	– 7 °C	25.4	28.9	32.4	38.2
	– 9 °C	25.5	31.3	37.0	46.6
	– 11 °C	31.7	-	-	-
	– 13 °C	-	-	-	-
Siedlisko (B)	Nonfrozen	25.9	29.7	33.5	40
	– 3 °C	27.3	31.3	35.3	41.9
	– 10 °C	21.1	39.4	-	-
	– 18 °C	-	-	-	-

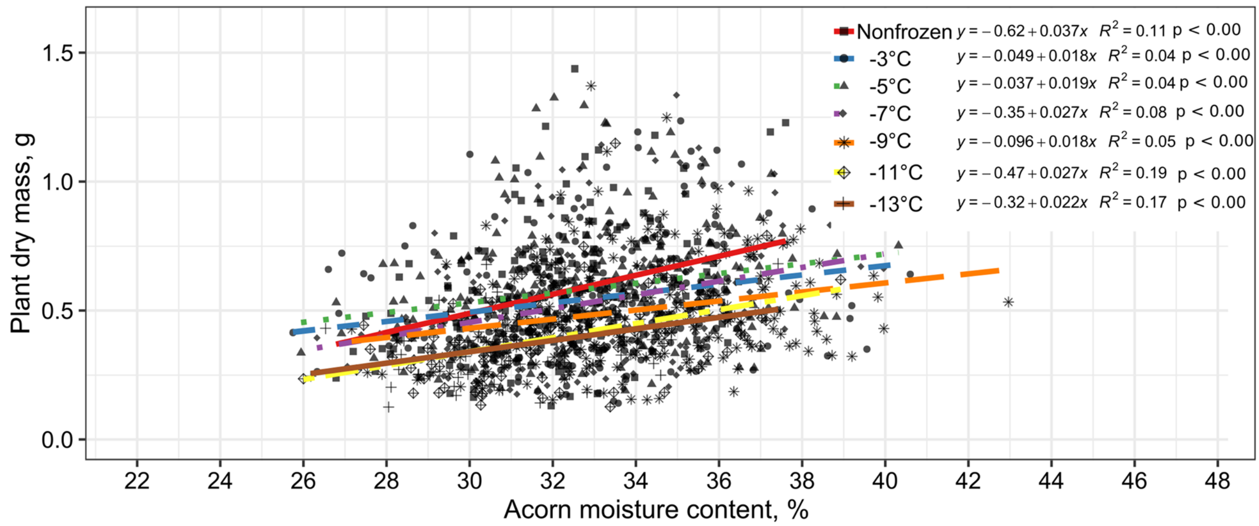

Fig. 3 Effect of drying of *Quercus robur* acorns (non-frozen) on the dry weight of derived 3-month-old seedlings in relation to freezing temperature: – 3°, – 5°, – 7°, – 9°, – 11° or – 13 °C. Non-frozen acorns from seed lot A stored at 3 °C were used as the control. Partial R^2 values are presented

study with our earlier observations (Chmielarz 1997), as well as the findings of studies performed on pedunculate oaks of Polish provenances using DTA methods (crystallization exotherms) (Suszka 2002), we conclude that seeds from all investigated origins have a frost resistance threshold of approximately – 10 °C.

Systematic studies on the storage of acorns conducted by Suszka and Tylkowski (1980) showed seed resistance to temperatures from – 1 °C to – 3 °C even during three years of storage. Acorns with a moisture content of 46% after 6 months of storage at – 4 °C germinated at a frequency of 40% (that before storage was 90%) in the study of Schönborn (1964). Zajceva (1950) demonstrated a lack of resistance of pedunculate oak seeds to temperatures below – 5 °C, while 0 °C was suggested as the optimal temperature for acorn storage. Holmes and Buszewicz (1956) pointed out that at a temperature of – 12 °C, seeds lost their viability in a very short time, regardless of seed moisture content. Schönborn (1964) tested a lower temperature and found that seeds desiccated in acorns to a moisture content of 25% lost their germinability when frozen to – 14 °C. Guthke (1993) observed the frost resistance of acorns under natural conditions and found that seedlings emerged from 60% of the seeds after winter when the ground temperature decreased periodically to – 9 °C, with an air temperature of – 15 °C. These results support the hypothesis of a threshold temperature for frozen acorns of approximately – 10 °C, which is also the temperature we observed in our experiment. Effective under mild freezing conditions (to a minimum of – 10 °C), a high acorn moisture content seems to prevent seeds from dying.

Similar thresholds can be found in other *Quercus* species. Boese et al. (1985) determined a threshold for *Q. velutina* Lam. of approximately – 7 °C, and other authors measuring the exotherm profiles for *Q. robur* L. seeds found a critical freezing temperature of approximately – 9 °C for acorns at a moisture content of approximately 42% (Chmielarz 1997). For other oak species, a threshold temperature for ice crystallization in seeds was found for *Q. pyrenaica* Willd. at – 4.8 °C, for *Q. faginea* Lam. at – 7.5 °C, for *Q. ilex* L. at – 9.5 °C and finally for *Q. coccifera* L. at – 14 °C. For these species, the results were consistent with those of polyelectrolyte leakage analysis, where Lt50 values arranged *Quercus* species in a very similar way (Esteso-Martínez and Gil-Pelegrín 2004).

4.2 Desiccation sensitivity

During desiccation of acorns, which contain recalcitrant seeds sensitive to desiccation, toxic oxidation products and free radicals are formed in the cotyledons earlier than in the embryonic axes (Finch-Savage et al. 1992). This feature has also been used during cryopreservation of the pedunculate oak axis apical meristem (plumule), where all other parts of the seed were cut off before plumule drying (Chmielarz et al. 2011). Suszka (2002) found that drying whole acorns to a moisture content below 38% decreased seedling emergence, and seeds of acorns dried to a moisture content below 22% did not germinate (Suszka, 2002). We observed that the drying of acorns decreased the 3-month-old seedling mass, regardless of the temperature applied. However, temperatures lower than – 10 °C could increase this effect. Lower seedling mass has further consequences because it is known (Grossnickle 2012) that smaller seedlings have a lower survival rate. It was also noticed that in our drying experiment, at the final stage of acorn drying to a moisture content of approximately 30%, greater dispersion of extreme individual moisture content values occurred for the whole dried sample compared with the differences observed at the beginning of drying. Finch-Savage (1992) suggested that in this critically low moisture content of acorns, all 'free' (unbound) cellular water is lost. Furthermore, he suggested that viability is primarily related to the moisture content of the cotyledons, which subsequently causes a decline in viability in the embryonic axis. As the acorn drying time increases, the average time required for seed germination and seedling emergence also increases (Chmielarz 1997). In our current experiments, seeds significantly started to lose their germinability and seedling emergence when desiccated to a moisture content of 31.0%. For the same seed lot, a moisture content of 32.9% was still safe. However, for the second seed lot studied here, characterized by a higher moisture content after harvesting, the critical moisture content was 35.7%, while a moisture content of 38.2% was still safe. This indicates that the critical moisture content at which oak seeds begin to lose their ability to germinate and produce seedlings can depend largely on their moisture content when they fall from the tree. Furthermore, the range of critical threshold moisture contents when seeds start to lose their germinability is very narrow. In both experiments, germination and seedling emergence laboratory tests showed some differences, especially in some critical variants, which reassured us that the seedling emergence test could be applied to assess the true viability of seeds in order to produce properly growing plants. However, it is important to note that in many experiments, seedling emergence assays are not performed.

4.3 To what extent can desiccation increase the frost resistance of recalcitrant seeds?

Research carried out on orthodox seeds (Chmielarz 2009, 2010b) or plumules of embryonic axes (Chmielarz et al. 2011) of recalcitrant seeds shows that the freezing sensitivity of seeds or their parts increases with an increased moisture content (Chandel et al. 1995; Tompsett and Pritchard 1998; Daws and Pritchard 2008). We found in our experiment that for whole pedunculate oak seeds, which belong to the recalcitrant category, a reduction in the moisture content does not always lead to higher freezing tolerance. At temperatures of − 3 °C and − 5 °C, drying to a moisture content below 40% (the optimum level for long-term storage) did not significantly improve seed survival. Our results strongly connect the loss of recalcitrant seed viability during cooling with a decrease in moisture content. However, slight drying of acorns to a moisture content below 40% improves seed germinability at temperatures of − 7 °C and − 9 °C. The positive effect of further acorn drying (down to a moisture content of 30% compared with a moisture content of 40%) was observed in our experiments at − 11 °C and − 13 °C. However, here, the loss of seed viability, because of freezing damage, exceeded the benefits of acorn drying.

Thus, whole recalcitrant seeds from pedunculate oaks respond to freezing temperatures in a desiccated state in a different way than seeds from the orthodox category. Similar observations were published by Xin et al. (2010) on the dehydration of intact *Q. acutissima* seeds. The authors showed that freezing sensitivity increased when seeds were dried compared with that of seeds with a higher moisture content. This drying behaviour is the opposite of that for *orthodox* seeds. We demonstrated in our earlier epigenetic research (Michalak et al. 2015) performed on acorns stored with an optimal moisture content at − 3 °C for up to 18 months that global demethylation is significantly related to seed ageing, and Finch-Savage claimed (1992) that rapid ageing in recalcitrant seeds is similar to desiccation damage, causing a loss of viability in oak seeds. Connor and Sowa (2002) reported that seeds of *Q. pagoda* and *Q. nigra*, which were dried prior to storage, had lower germination than those stored fully hydrated. Pammenter et al. (1994) concluded that the inability to dry recalcitrant seeds might be due to a breakdown in the coordination of metabolism, leading to uncontrolled free-radical-mediated oxidative damage. Other authors claimed that acorns of pedunculate oak adjusted to a moisture content of 46% and stored for 6 months had the highest germination at 76%, while the controls and those adjusted to a moisture content of 37% had the lowest value of 17% (Özbingöl and O'reilly 2005). We can confirm the difficulties of drying pedunculate oak seeds, especially in the context of increasing their freezing tolerance.

5 Conclusions

Our study focused on determining the frost resistance of partially desiccated pedunculate oak seeds frozen at different temperatures down to − 18 °C for 2 weeks. The results demonstrated that there are many factors determining the survival of pedunculate oak seeds at temperatures below 0 °C. These factors include the weather conditions during seed maturation on a tree and, as a consequence, the moisture content of the acorns when falling from the tree, as well as the acorn moisture content during freezing and, finally, the freezing temperature. The survival benefit of seed drying during cooling below 0 °C is very limited. This indicates that for pedunculate oak seeds, the rule that is obvious for orthodox seeds—the lower the moisture content is, the higher the frost resistance—does not apply. Our research showed that only a slight reduction in acorn moisture content below 40% increases seed survival at − 7 °C or − 9 °C. We also identified the limiting temperature (− 10 °C) below which pedunculate oak seeds rapidly lose their ability to germinate and emerge. It was also observed that the severe drying and freezing of acorns, interacting together or separately, can lead to abnormal seedlings, characterized by a significantly lower dry mass.

6 Appendix

Table 4 Full model results for germination and seedling emergence of oak seeds presented in Table 1. Analysis of deviance results for germination percentage and seedling emergence in controlled conditions at $p < 0.05$

Seed lot	Temperature	Factor	Germination				Seedling emergence			
			df	Dev.	Resid. Dev.	Pr(>chi)	*df*	Dev.	Resid. Dev.	Pr(>chi)
Czmoń (A)	Nonfrozen	Null			144.32				145.32	
		MC	5	90.97	23.35	0.000	5	128.13	17.19	0.000
	− 3 °C	Null			140.61				198.27	
		MC	5	128.60	12.00	0.000	5	184.68	13.59	0.000
	− 5 °C	Null			135.55				194.61	
		MC	5	120.91	14.65	0.000	5	187.90	6.71	0.000
	− 7 °C	Null			116.55				131.28	
		MC	5	108.46	8.09	0.000	5	118.44	12.84	0.000
	− 9 °C	Null			146.55				156.77	
		MC	5	124.73	21.82	0.000	5	138.42	18.35	0.000
	− 11 °C	Null			64.75				57.65	
		MC	5	54.52	10.13	0.000	5	47.50	10.15	0.000
	− 13 °C	Null			54.25				40.42	
		MC	5	37.62	16.63	0.000	5	23.34	17.08	0.002
Siedlisko (B)	Nonfrozen	Null			144.45				120.66	
		MC	8	102.06	42.39	0.000	8	82.36	38.30	0.000
	− 3 °C	Null			144.06				133.52	
		MC	8	113.70	30.36	0.000	8	110.54	22.98	0.000
	− 10 °C	Null			223.55				207.23	
		MC	8	171.51	52.04	0.000	8	166.40	40.83	0.000
	− 18 °C	Null	-	-	-	-	-	-	-	-
		MC	-	-	-	-	-	-	-	-

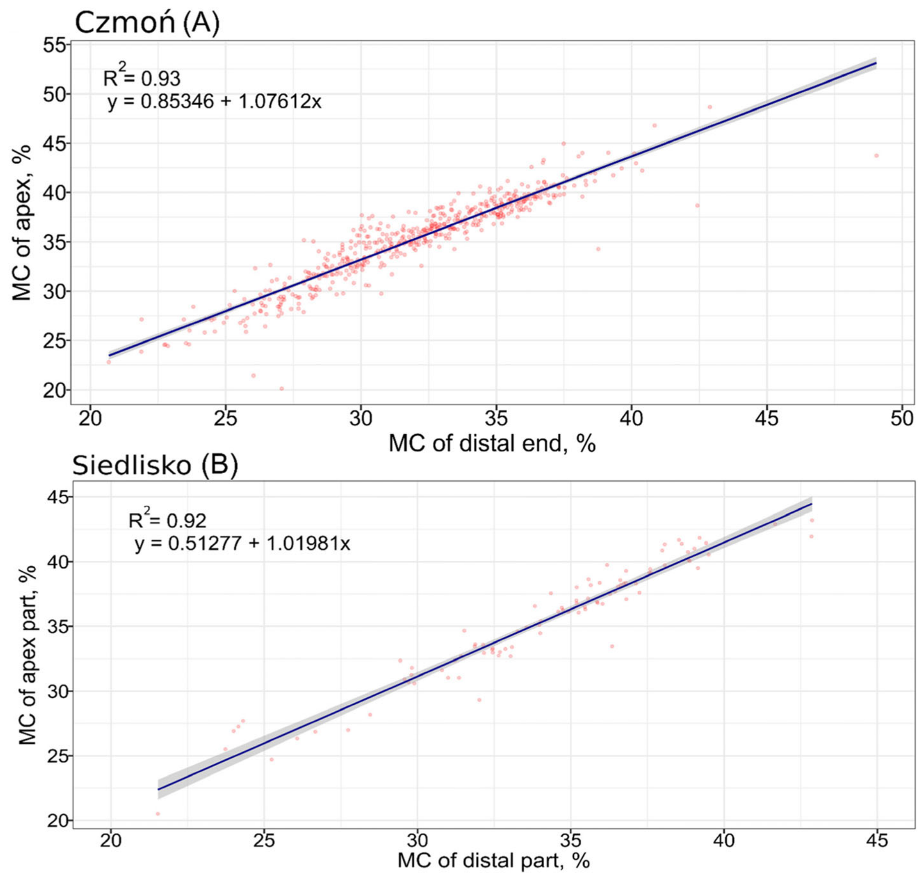

Fig. 4 Linear model of moisture content of distal and apex parts of acorns. For seed lot **A** and **B**

Acknowledgements
The authors would like to thank Mrs Elżbieta Nogajewska, Danuta Szymańska, Magdalena Sobczak, Agata Obarska and Paulina Pilarz for technical assistance in conducting all experiments.

Authors' contributions
Conceptualization: P Chmielarz, J Suszka, MK Wawrzyniak; Methodology: P Chmielarz, J Suszka, MK Wawrzyniak; Formal analysis and investigation: J Suszka, MK Wawrzyniak; Data visualization: MK Wawrzyniak; Writing – original draft preparation: P Chmielarz; Writing – review and editing: P Chmielarz, J Suszka, MK Wawrzyniak; Funding acquisition: P Chmielarz; Resources: J Suszka; Supervision: P Chmielarz. The authors read and approved the final manuscript.

Funding
This study was funded by the General Directorate of State Forests in Warsaw, Poland (EO.271.3.7.2018). Additional support was provided by the Institute of Dendrology Polish Academy of Sciences.

Availability of data and materials
The datasets generated and analyaed during the current study are available in the Figshare repository (https://doi.org/10.6084/m9.figshare.16837747).

Declarations

Ethics approval and consent to participate
The authors declare that the study was not conducted on endangered, vulnerable or threatened species.

Consent for publication
All authors gave their informed consent to this publication and its content.

Competing interests
The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Received: 16 July 2021 Accepted: 24 November 2021
Published online: 24 February 2022

References

Berjak P, Pammenter NW (2000) What ultrastructure has told us about recalcitrant seeds. Rev Bras de Fisiolog Veg 12:22–55

Berjak P, Walker M, Watt MP, Mycock DJ (1999) Experimental parameters underlying failure or success in plant germplasm cryopreservation: a case study on zygotic axes of *Quercus robur* L. Cryo-letters 20:251–262

Boese SR, George MF, Mitchell RJ, Martin U, Mc Quilkin RA (1985) Physical aspects of freezing in black oak acorns. In: Proceedings of the Fifth Central Hardwood Forest Conference. Urbana-Champaign, Illinois, pp 222–227

Chandel K (1995) Desiccation and Freezing Sensitivity in Recalcitrant Seeds of Tea, Cocoa and Jackfruit. Ann Bot 76(5):443–450. https://doi.org/10.1006/anbo.1995.1118

Chmielarz P (1997) Frost resistance of *Quercus robur* L. acorns. In: Treatment and storage of oak seeds. Present situation and research. Mitt.Biol.Bundesanst. Land-Forstwirtsch, Berlin-Dahlem, H.329, pp 76–81

Chmielarz P (2007) Kriogeniczne przechowywanie nasion leśnych drzew liściastych z kategorii orthodox i suborthodox (intermediate) [Cryogenic seed storage of orthodox and suborthodox (intermediate) deciduous forest trees species] In Polish. Bogucki, Poznań, Poland, pp. 1-143.

Chmielarz P (2009) Cryopreservation of dormant European ash (*Fraxinus excelsior*) orthodox seeds. Tree Physiol 29(10):1279–1285. https://doi.org/10.1093/treephys/tpp064
Chmielarz P (2010a) Cryopreservation of conditionally dormant orthodox seeds of *Betula pendula*. Acta Physiol Plant 32(3):591–596. https://doi.org/10.1007/s11738-009-0437-6
Chmielarz P (2010b) Cryopreservation of dormant *orthodox* seeds of European hornbeam (*Carpinus betulus*). Seed Sci Technol 38(1):146–157. https://doi.org/10.15258/sst.2010.38.1.15
Chmielarz P, Michalak M, Pałucka M, Wasileńczyk U (2011) Successful cryopreservation of Quercus robur plumules. Plant Cell Rep 30(8):1405–1414. https://doi.org/10.1007/s00299-011-1049-3
Chmielarz P, Walters C (2007) Desiccation sensitivity of white and black (red) oak embryonic axes. S African J Bot 73(3):498. https://doi.org/10.1016/j.sajb.2007.04.042
Connor KF, Sowa S (2002) Recalcitrant behaviour of temperate forest tree seeds: storage, biochemistry and physiology. In: Proceedings of the eleventh biennial southern silvicultural research conference, General Technical Report, SRS-48
Connor KF, Sowa S (2003) Effects of desiccation on the physiology and biochemistry of *Quercus alba* acorns. Tree Physiol 23(16):1147–1152. https://doi.org/10.1093/treephys/23.16.1147
R Core Team (2021) R: A Language and Environment for Statistical Computing. https://www.R-project.org/
Crawley MJ (2013) The R book. Chichester, Wiley
Daws MI, Pritchard HW (2008) The Development and Limits of Freezing Tolerance in *Acer pseudoplatanus* Fruits Across Europe is Dependent on Provenance. Cryo-letters 29(3):189–198
Domisch T, Martz F, Repo T, Rautio P (2018) Winter survival of Scots pine seedlings under different snow conditions. Tree Phys 38(4):602–616. https://doi.org/10.1093/treephys/tpx111
Ellis RH, Hong TD, Roberts EH (1991) Effect of storage temperature and moisture on the germination of papaya seeds. Seed Sci Res 1(1):69–72. https://doi.org/10.1017/s0960258500000659
Esteso-Martínez J, Gil-Pelegrín E (2004) Frost resistance of seeds in Mediterranean oaks and the role of litter in the thermal protection of acorns. Ann For Sci 61(5):481–486. https://doi.org/10.1051/forest:2004042
Field A, Miles J, Field Z (2012) Discovering statistics using R. SAGE Publications
Finch-Savage WE (1992) Embryo Water Status and Survival in the Recalcitrant Species *Quercus robur* L: Evidence for a Critical Moisture Content. J Exp Bot 43(5):663–669. https://doi.org/10.1093/jxb/43.5.663
Giertych MJ, Suszka J (2010) Influence of cutting off distal ends of *Quercus robur* acorns on seedling growth and their infection by the fungus *Erysiphe alphitoides* in different light conditions. Dendrobiology 64: 73-77
Giertych MJ, Suszka J (2011) Consequences of cutting off distal ends of cotyledons of *Quercus robur* acorns before sowing. Annals Forest Sci 68(2): 433–442. https://doi.org/10.1007/s13595-011-0038-6
Gordon AG (1992) Seed storage. In: Gordon AG (ed) Seed Manual for Forest Trees. HMSO, Forestry Commission, London, pp 98–104
Gosling PG (1989) The Effect of Drying *Quercus robur* Acorns to Different Moisture Contents, followed by Storage, either with or without Imbibition. Forestry (Lond) 62(1):41–50. https://doi.org/10.1093/forestry/62.1.41
Grossnickle SC (2012) Why seedlings survive: influence of plant attributes. New Forests 43(5-6):711–738. https://doi.org/10.1007/s11056-012-9336-6
Guerrant EO, Havens K, Maunder M (eds) (2004) *Ex situ* plant conservation: supporting species survival in the wild. Island Press, Washington, pp 139–161
Guthke J (1993) Langzeitlagerung von Eichensaatgut. Probleme und Moeglichkeiten [Storage of acorns. Problems and possibilities], Doctoral dissertation, Hannover Universitat, Hannover Germany
Holmes GD, Buszewicz G (1956) Longevity of acorns with several storage methods. Rep For Res For Comm, London, pp 88–94
Hong TD, Linington S, Ellis RH (1998) Compendium of information on seed storage behaviour https://CRAN.R-project.org/package=emmeans
Kreyling J, Henry HA (2011) Vanishing winters in Germany: soil frost dynamics and snow cover trends, and ecological implications. Climate Res 46(3):269–276. https://doi.org/10.3354/cr00996
Lenth RV (2022) emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.7.2. https://CRAN.R-project.org/package=emmeans
Michalak M, Plitta-Michalak BP, Naskręt-Barciszewska M, Barciszewski J, Bujarska-Borkowska B, Chmielarz P (2015) Global 5-methylcytosine alterations in DNA during ageing of *Quercus robur* seeds. Ann Bot 116(3):369–376. https://doi.org/10.1093/aob/mcv104
Özbingöl N, O'reilly C (2005) Increasing acorn moisture content followed by freezing-storage enhances germination in pedunculate oak, Forestry: An International Journal of Forest Research 78(1):73–81. https://doi.org/10.1093/forestry/cpi007
Pammenter NW, Berjak P, Farrant JM, Smith MT, Ross G (1994) Why do stored hydrated recalcitrant seeds die? Seed Sci Res 4(2):187–191. https://doi.org/10.1017/s0960258500002178
Poulsen KM, Eriksen EN (1992) Physiological aspects of recalcitrance in embryonic axes of *Quercus robur* L. Seed Sci Res 2(4):215–221. https://doi.org/10.1017/s0960258500001409
Pritchard HW (2004) Classification of seed storage 'types' for *ex situ* conservation in relation to temperature and moisture. In: Guerrant EO, Havens K, Maunder M (eds) *Ex situ* plant conservation: supporting species survival in the wild. Island Press, Washington, pp 139–161
Ranal MA, de Santana DG (2006) How and why to measure the germination process? Brazilian J Bot 29(1):1–11. https://doi.org/10.1590/S0100-84042006000100002
Reinmann AB, Susser JR, Demaria EMC, Templer PH (2019) Declines in northern forest tree growth following snowpack decline and soil freezing. Glob Change Biol 25(2):420–430. https://doi.org/10.1111/gcb.1442
Renard SM, Mc Intire EJ, Fajardo A (2016) Winter conditions – not summer temperature – influence establishment of seedlings at white spruce alpine treeline in Eastern Quebec. J Veg Sci 27(1):29–39. https://doi.org/10.1111/jvs.12347
Roberts EH (1973) Predicting the storage life of seeds. Seed Sci Tech 1:499–514
Schönborn A (1964) Die Aufbewahrung des Saatgutes der Waldbäume [Storage of forest tree seeds]. BLV Verlagsgesellschaft, München
Schroder T (2002) On the geographic variation of *Ciboria batschiana* (Zopf) Buchwald, the main pathogenic fungus on acorns of *Quercus robur* and *Q. petraea* in Europe. Dendrobiology 47:13-19
Schroeder WR, Walker DS (1987) Effects of Moisture Content and Storage Temperatures on Germination of *Quercus macrocarpa* Acorns. J Environ Hort 5(1):22–24. https://doi.org/10.24266/0738-2898-5.1.22
Subbiah A, Ramdhani S, Pammenter NW, Macdonald AHH, Sershen (2019) Towards understanding the incidence and evolutionary history of seed recalcitrance: An analytical review. Perspect Plant Ecol Ev Syst 37:11–19. https://doi.org/10.1016/j.ppees.2019.01.001
Suszka B, Muller C, Bonnet-Masimbert M (1996) Seeds of forest broadleaves: from harvest to sowing. INRA, Paris
Suszka B, Tylkowski T (1980) Storage of acorns of English oak (*Quercus robur* L.) over 1-5 winters Arb. Kórnickie 25:199–230
Suszka J (2002) Improvement of methods of storage and pre-sowing treatment seeds of English oak (*Quercus robur* L.) and European beech (*Fagus sylvatica* L.). Doctoral dissertation, Institute of Dendrology Polish Academy of Sciences, Kórnik, Poland
Tompsett P (1998) The Effect of Chilling and Moisture Status on the Germination, Desiccation Tolerance and Longevity of *Aesculus hippocastanum* L. Seed. Ann Bot 82(2):249–261. https://doi.org/10.1006/anbo.1998.0676
Wawrzyniak M, Suszka J, Chmielarz P (2021) Desiccation and frost resistance of penduncalate oak (Quercus robur L.) individual seeds. figshare. [Dataset]. https://doi.org/10.6084/m9.figshare.16837747.v1
Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. https://doi.org/10.1007/978-3-319-24277-4
Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen T, Miller E, Bache S, Müller K, Ooms J, Robinson D, Seidel D, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019) Welcome to the tidyverse. J Open Source Soft 4(43): 1686. https://doi.org/10.21105/joss.01686
Xin X, Wang WJ, Yin GK, Lin J, Lu XX, Jing XM (2010) Freezing sensitivity and low temperature storage of *Quercus acutissima* and *Quercus variabilis* seeds. Seed Sci Technol 38(1):170–183. https://doi.org/10.15258/sst.2010.38.1.17
Yeo SR, Kim W, Kim KY (2017) Eurasian snow cover variability in relation to warming trend and Arctic Oscillation. Clim Dyn 48(1-2):499–511. https://doi.org/10.1007/s00382-016-3089-4
Zaitseva AA (1950) Zimneekhranenie semennykh zheludeĭ [Cold storage of oak seeds]. Lesn Khoz 10:63–72

Publisher's Note